IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :

U.S. National Serial No. :

Filed :

PCT International Application No. : PCT/EP03/08796

## VERIFICATION OF A TRANSLATION

I, Charles Edward SITCH BA,

Deputy Managing Director of RWS Group Ltd UK Translation Division, of Europa House, Marsham Way, Gerrards Cross, Buckinghamshire, England declare:

That the translator responsible for the attached translation is knowledgeable in the German language in which the below identified international application was filed, and that, to the best of RWS Group Ltd knowledge and belief, the English translation of the international application No. PCT/EP03/08796 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.

Date: January 7, 2005

Signature : C Sitch

For and on behalf of RWS Group Ltd

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England.

Replaced by Article 34



**Claims**

1. A method for detecting disseminated tumor cells from a body fluid, in which
(a) tumor cells are enriched by a cell separation medium which has a density in the range from 1.055 to 1.065 g/ml being overlaid with the body fluid and being centrifuged; and
(b) it is determined whether the enriched cells express an epithelial marker.

2. The method as claimed in claim 1, characterized in that the epithelial marker is a cytokeratin.

3. The method as claimed in claim 2, characterized in that the cytokeratin is selected from the group consisting of cytokeratin 1 to 20.

4. The method as claimed in any of the preceding claims, characterized in that cytokeratin-positive and cytokeratin-negative blood cells are separated from one another, with the enriched tumor cells being present in the same fraction as the cytokeratin-negative blood cells.

5. The method as claimed in any of the preceding claims, characterized in that non-tumor cells which express at least one of cytokeratins 1-20 are separated from tumor cells which express at least one of cytokeratins 1-20.

6. The method as claimed in any of the preceding claims, characterized in that the centrifugation is carried out in a vessel which is divided by a porous barrier, a filter, a sieve or a flap into an upper and a lower compartment, the cell separation medium being introduced into the lower compartment, and the body fluid being put in the upper compartment.

7. The method as claimed in claim 6, characterized in that the porous barrier, the filter, the sieve or the flap have a thickness of 0.5-10 mm, preferably of 1-5 mm.

8. The method as claimed in claim 6 or 7, characterized in that the porous barrier, the filter, the sieve or the flap have a porous size of 20-100 µm, preferably 20-30 µm.

9. The method as claimed in any of claims 6 to 8, characterized in that the porous barrier, the filter, the sieve or the flap consist of a hydrophobic material or are coated with a hydrophobic material.

10. The method as claimed in any of the preceding claims, characterized in that the cell separation medium comprises a dye which makes the cell separation medium distinguishable in color from the overlying body fluid, and thus simplifies location of the interphase.

11. The method as claimed in any of the preceding claims, characterized in that in step b) there is determination in single or combination analysis of whether the enriched cells express at least one epithelial marker such as, for example, one of cytokeratins 1-20.

12. The method as claimed in any of claims 1 to 11, characterized in that the determination of whether the enriched cells express an epithelial marker such as, for example, a cytokeratin comprises reverse transcription of mRNA from the enriched cells, and carrying out a PCR with at least one primer specific for an epithelial marker, such as, for example, cytokeratin-specific.

13. The method as claimed in any of claims 1 to 11, characterized in that the enriched cells are brought into contact with a monoclonal antibody which is specific for a particular epithelial marker such as, for example, a particular cytokeratin, and in step b) there is determination in single or combination analysis of whether the enriched cells express at least one epithelial marker such as, for example, one of cytokeratins 1-20.

14. A kit comprising a cell separation medium which has a density in the range 1.055-1.065 g/ml, and means for detecting the expression of at least one epithelial marker.

15. The kit as claimed in claim 14, characterized in that the epithelial marker is cytokeratin.

16. The kit as claimed in claim 14 or 15, characterized in that it comprises means for detecting the expression of at least one of cytokeratins 1-20.

17. The kit as claimed in claim 15 or 16, characterized in that the means for detecting the expression of cytokeratin is selected from the group consisting of monoclonal anti-cytokeratin antibodies and cytokeratin-specific primers.

18. The kit as claimed in any of claims 14 to 17, characterized in that a washing buffer, optionally in concentrated form, is additionally present for washing the enriched cells.

19. The kit as claimed in any of claims 14 to 18, characterized in that at least one centrifugation vessel is additionally present.